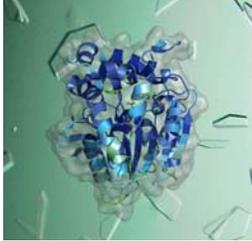


**Cif: A *Pseudomonas* Epoxide-Hydrolase Virulence Factor**



Dean R. Madden  
Dartmouth Medical School



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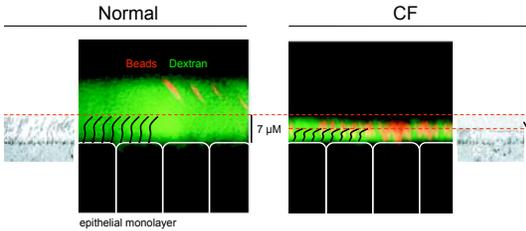
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**Airway Epithelium**



Normal CF

7  $\mu$ M

epithelial monolayer

adapted from Matsui et al. (1998) Cell 95:1005

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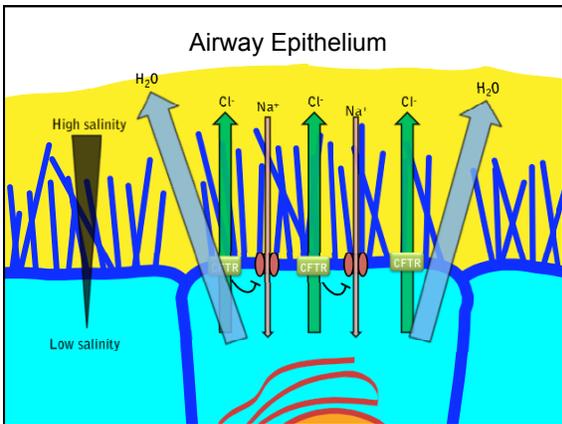
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**Airway Epithelium**



High salinity Low salinity

H<sub>2</sub>O Cl<sup>-</sup> Na<sup>+</sup> Cl<sup>-</sup> Na<sup>+</sup> Cl<sup>-</sup> H<sub>2</sub>O

TRP CFTR CFTR

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*Pseudomonas aeruginosa* is a leading cause of opportunistic lung infection

- Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the world, and *P. aeruginosa* infection is especially common in severe cases.
- *P. aeruginosa* is the leading gram-negative cause of ventilator associated pneumonia (VAP), and has a mortality rate of 34% to 68%.
- Community-Acquired Pneumonia (CAP) caused by *P. aeruginosa* is extremely difficult to treat, and has a 55% mortality rate.

Wise & Tashkin (2007) *Am J Med*  
 El Solh et al. (2008) *Am J Respir Crit Care Med*  
 Garcia-Vidal et al. (2007) *Eur Respir J*

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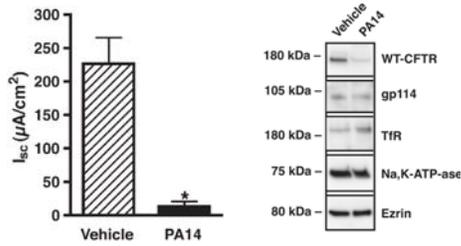
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*P. aeruginosa* secretes a factor that reduces chloride ion efflux



Swiatecka-Urban et al. (2006) *AJP Cell*

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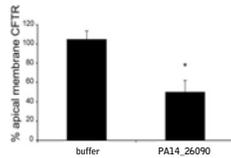
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Cif targets CFTR

- The gene product of PA14\_26090 is secreted
- Purified PA14\_26090 protein causes the removal of CFTR from the apical membrane of airway epithelial cells
- PA14\_26090 was re-named CFTR Inhibitory Factor (Cif)



adapted from MacEachran et al. (2007) *Infect Immun*

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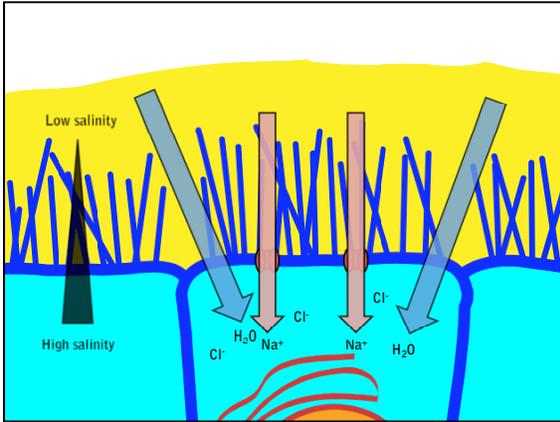
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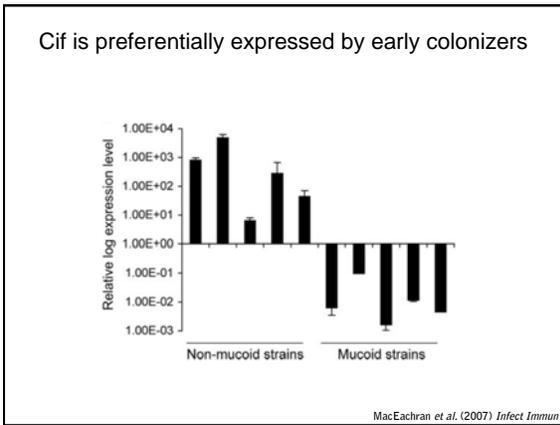
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How does Cif work?

- What is Cif's physiological function?

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A candidate epoxide hydrolase...

- Related to an enzyme from an ECH-degrading *Pseudomonad*
- Homologous to  $\alpha/\beta$  hydrolase superfamily
- Has 25% sequence identity to multiple known epoxide hydrolases
- Sequence alignment predicted a highly conserved active site histidine residue at position 297

MacEachran et al. (2007) *Infect Immun.* 75:3902

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...with caveats...

- Borderline sequence identity (~25%).
- Comparable level of sequence identity to other  $\alpha/\beta$  hydrolases (lysophospholipase, CC-hydrolase).
- Conserved active-site motifs are missing (proton-donating Tyr, oxyanion HGxP).
- Authentic substrates are unknown.

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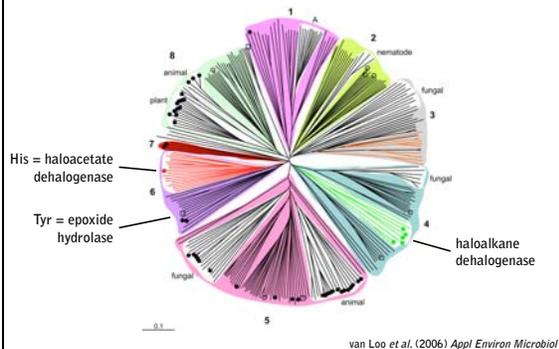
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... and phylogenetic ambiguity




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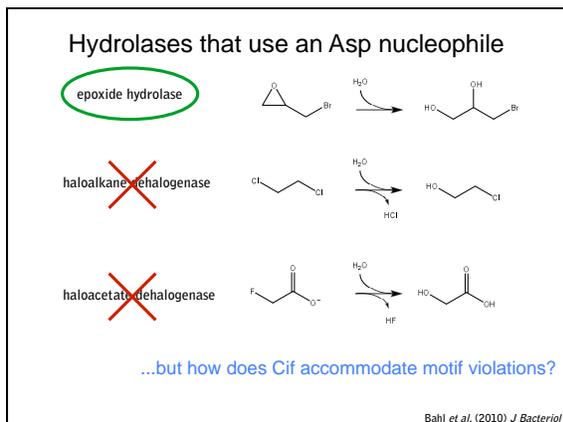
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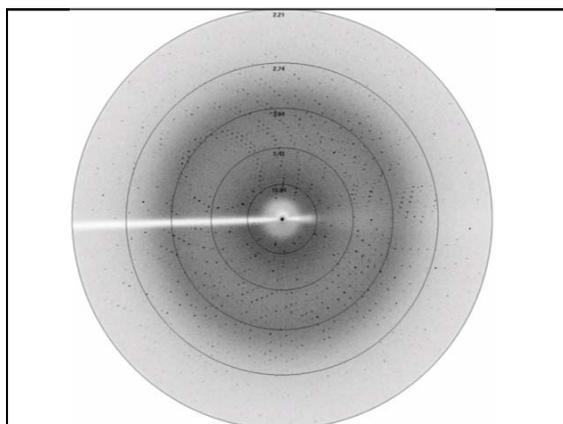
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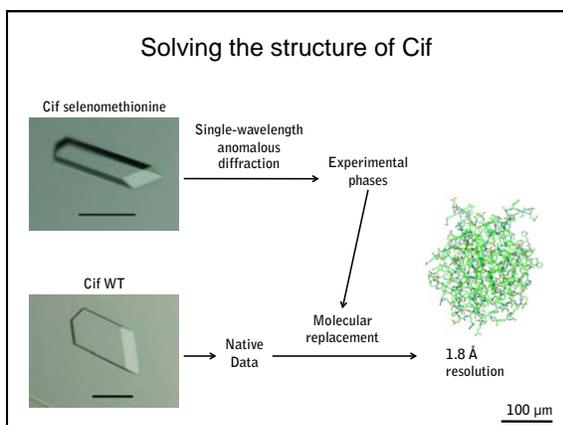
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Cif WT <sup>a</sup>	
<b>Data Collection</b>	
Space Group	C2
Unit cell dimensions:	
<i>a,b,c</i> (Å)	168.2, 83.9, 89.0
$\beta$ (°)	100.5
Resolution	1.80 Å
$R_{\text{sym}}$ (%)	7.4 (27.6)
$I/\sigma I$	19.49 (5.16)
Completeness (%)	99.8 (99.9)
<b>Refinement</b>	
Total number of reflections	112388
Reflections in the test set	5733
$R_{\text{work}}/R_{\text{free}}$	15.98/18.39
Number of waters	881
Bond length RMSD	0.007
Bond angle RMSD	1.040

a. high resolution shell is 1.87-1.80 Å

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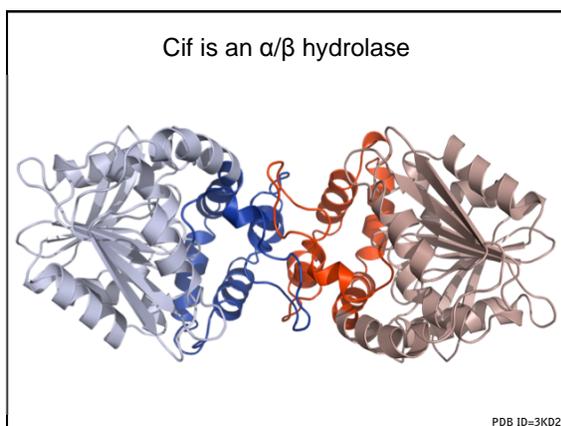
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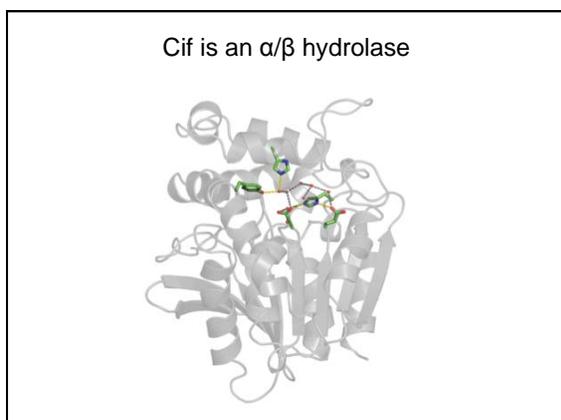
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## How does Cif work?

- Cif is an epoxide hydrolase
- Cif is the founding member of a class of pathogen EHs that use a Tyr/His ring-opening pair
- Is EH activity required for CFTR inhibition?

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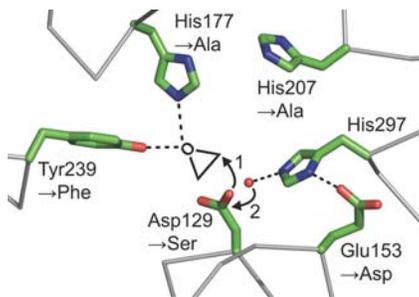
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## Cif active site and EH mechanism




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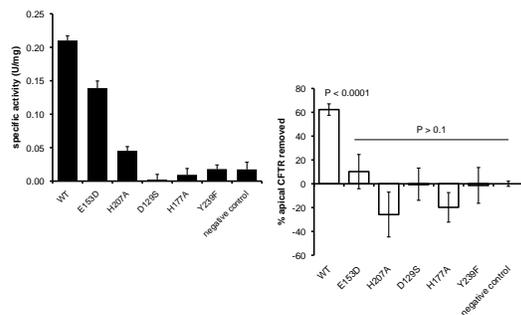
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## EH activity is required for CFTR inhibition



Jen Bomberger

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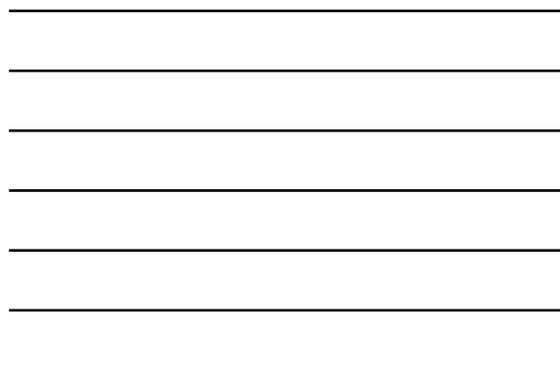
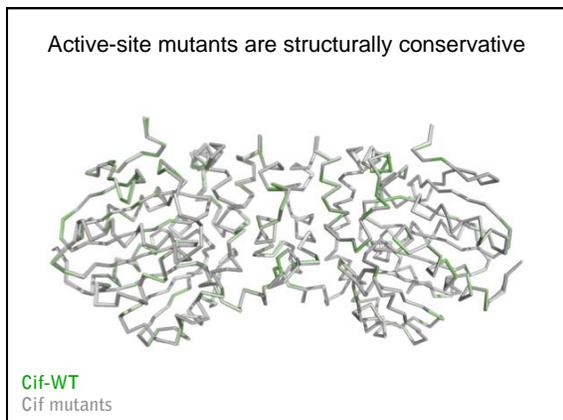


Table 1. Data Collection and Refinement Statistics

	D129S	E153Q	E153Q -EBH	E153D	Y239F	H177A	H177Y	H207A	H269A
Space Group	C2								
Unit cell dimensions ( $\text{\AA}$ )	108.2, 94.0, 99.2	108.4, 93.9, 99.5	108.1, 94.0, 99.5	108.4, 94.1, 99.2	108.7, 94.0, 99.3	107.8, 93.8, 99.0	108.4, 94.1, 99.3	108.8, 93.8, 99.5	108.1, 93.6, 99.8
Resolution ( $\text{\AA}$ )	1.55	1.66	1.90	1.36	1.50	2.12	1.50	1.90	1.50
$R_{\text{int}}$ (%)	5.6 (28.4)	9.0 (56.4)	9.0 (27.8)	7.5 (45.4)	5.8 (30.2)	9.7 (38.2)	5.1 (34.1)	7.0 (33.6)	3.9 (27.2)
Completeness (%)	98.0 (96.7)	97.1 (95.8)	99.4 (99.4)	98.7 (97.5)	97.2 (95.8)	99.8 (99.7)	99.8 (99.2)	97.3 (96.3)	99.7 (99.6)
<b>Molecular Replacement</b>									
Rotation function search									
LLG	2480	876	4994	5187	508	4980	2312	5089	4470
Z-score	32.3	30.5	33.8	35.2	35.3	34.0	31.5	32.4	24.6
Translation function search									
LLG	9551	9372	8755	9588	9968	9657	10028	9573	7647
Z-score	87.8	85.1	86.1	74.9	88.1	85.1	81.7	86.0	72.9
Overall log likelihood gain	16287	14795	12892	15185	16738	11853	16891	13780	12256
<b>Refinement</b>									
Total number of reflections	172879	184842	112809	258287	198239	48795	193152	140589	192075
Reflections in the test set	8644	7028	5707	12918	9895	2454	9794	6796	9636
$R_{\text{work}}/R_{\text{free}}$ (%)	15.9/17.6	18.1/20.8	18.9/22.4	21.2/22.3	16.3/18.2	16.6/20.6	16.4/18.2	16.2/19.8	16.9/18.3
Protein	9051	9098	9509	9451	9588	9580	9628	9495	9582
Reference	1224	876	829	907	1295	595	1244	828	1111
Other	0	0	0	0	0	0	0	0	0
Ramachandran plot (%)	91.6/8.0/0.0/0.0	91.3/8.3/0.4/0.0	91.8/5.0/0.0	90.9/5.0/0.0	91.3/8.3/0.4/0.0	91.3/8.3/0.4/0.0	91.8/2.0/0.0	91.2/8.0/0.0	91.0/8.0/0.0/0.0
$R_{\text{p}}$ ( $\text{\AA}$ )									
Protein	12.9	14.4	12.6	12.9	12.3	19.0	13.1	15.5	13.4
Bond length	0.006	0.006	0.006	0.006	0.006	0.007	0.006	0.006	0.005
Bond angle RMSD	1.083	1.023	1.022	1.062	1.080	0.994	1.082	1.067	1.066

\*Values in parentheses are for data in the highest resolution shell.  
 $R_{\text{p}} = \sum |F_{\text{obs}} - F_{\text{calc}}| / \sum F_{\text{obs}}$ , where  $F_{\text{obs}}$  and  $F_{\text{calc}}$  values are the  $h$ - $k$ - $l$  and mean measurements of the intensity of reflections.  
 $R_{\text{free}}$  is a robust indicator of the agreement of structure factors of symmetry-related reflections and is described in detail in Diederichs & Kappas (1997).  
 $R_{\text{p}} = \sum |F_{\text{obs}} - F_{\text{calc}}| / \sum F_{\text{obs}}$ , for the test set.  
 $R_{\text{free}} = \sum |F_{\text{obs}} - F_{\text{calc}}| / \sum F_{\text{obs}}$ , for the test set.  
 $R_{\text{p}} = \sum |F_{\text{obs}} - F_{\text{calc}}| / \sum F_{\text{obs}}$ , for the test set.



## How does Cif work?

- Cif is an epoxide hydrolase
- Cif is the founding member of a class of pathogen EHS
- EH activity is necessary for CFTR inhibition, but not sufficient.
- Is there an endogenous substrate?

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## Biological roles of epoxides

- Detoxification/metabolism
  - Environmental toxins
  - Reactive metabolic products
- Regulatory signals:
  - Blood pressure (sEHI)
  - Inflammatory response/ARDS (leukotoxin)
  - Pain
- epoxyeicosatrienoic acids (EETs) (reduce COX)
- epoxyoctadecenoic acids (EpOMES) (cytotoxic diols)

Decker (2009) *Arch. Toxicol.* **83**:297

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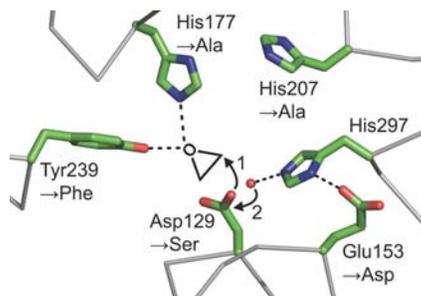
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## Cif active site and EH mechanism




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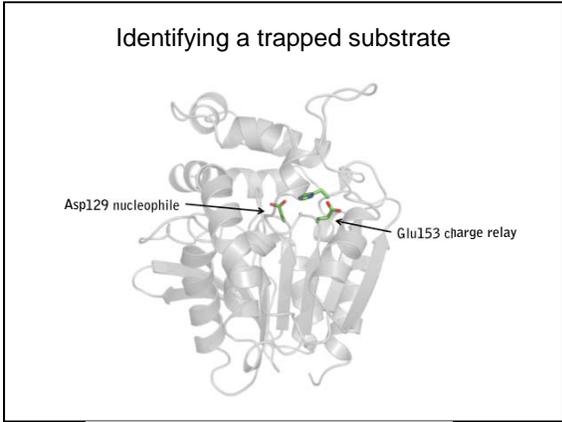
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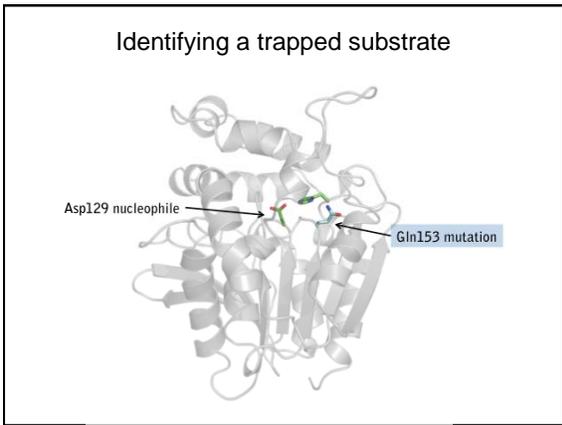
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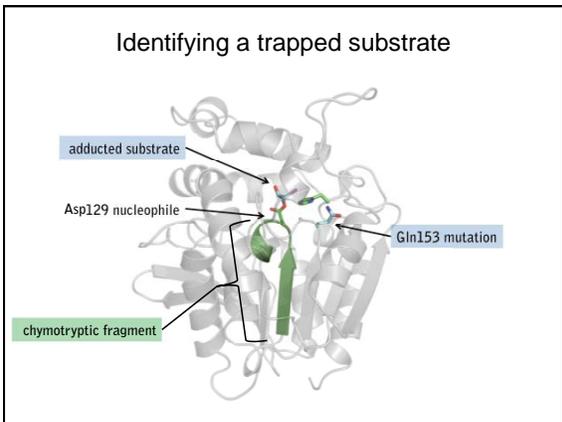
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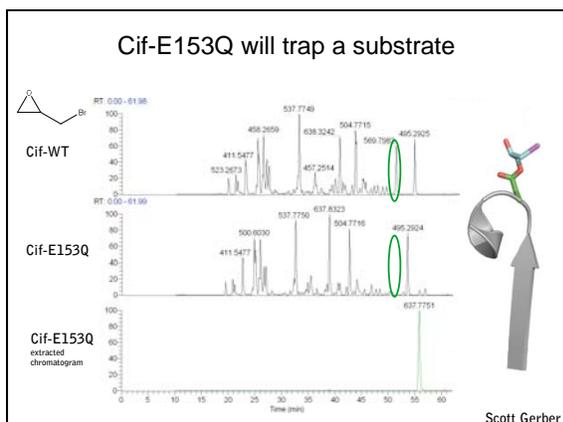
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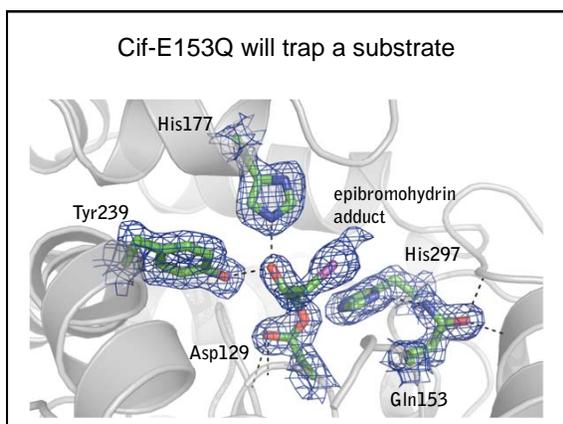
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**How does Cif work?**

- Cif is an epoxide hydrolase
- Cif is the founding member of a class of pathogen EHs
- EH activity is necessary for CFTR inhibition, but not sufficient.
- The E153Q mutation can be used as bait to trap candidate endogenous substrates that regulate CFTR expression.

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